Analysis of Anabolic Steroids

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1. Background

Steroids come in two forms which are anabolic and androgenic. Anabolic steroids promote muscle growth, enhance athletic or other physical performance and improve physical appearance. Androgenic steroids promote the development of sex characteristics and maintain traits such as body hair, deepening of the voice and baldness. Regardless of their specific physiological role, all steroids conatn the same basic cyclopentanoperhydrophenanthrene ringed nucleus. The variation on the basic nucleus determines the specific steroid and its properties. Steroids are most commonly found as injectable oils, tablets and capsules. Other forms may include powders, creams, liquid drops and transdermal patches.

2. Objective

The objective of this SOP is to establish guidelines to be used for the analysis of a sample that may contain an anabolic steroid.

3. Scope

This SOP is to be used by the laboratory staff of the Division of Analytical Chemistry at William A. Hinton State Laboratory Institute in Boston, MA.

4. Responsibility

Chemists are responsible for acquiring glassware, preparing chemical reagents and standards, sample analysis, and reporting. Chemists also perform instrument calibrations, maintenance and troubleshooting, ordering of supplies and other necessary tasks related to this analysis.

Laboratory Supervisors ensure that chemists are following this SOP. They may perform the duties of the chemists and must review raw data and reports generated by chemists. The Supervisor may advise the chemists of alternative testing methods. They ensure that quality control measures are within acceptable limits and determine when corrective actions are needed. They coordinate proficiency testing (PT), reporting and distribution of PT results. They oversee sample results distribution to outside agencies.

Directors ensure that the SOP is being followed and reviewed on a regular basis. They provide approval of standard operating procedures and review quality control documentations.

1. Related Documents

Cole, Michael, "The Analysis of Controlled Substances," London: John Wiley & Sons Ltd., 2003
Drug Enforcement Administration, "Basic Training Program for Forensic Drug Chemists," Drug Enforcement Administration.
Mills III, Terry et al, "Instrumental Data for Drug Analysis," 3rd ed., 6 vols., New York: CRC Press, 2006.
Moffat, A.C. et al, "Clarke's Isolation and Identification of Drugs," 2nd ed., London: The Pharmaceutical Press, 1986.
Moffat, A.C. et al. "Clarke's Analysis of Drugs and Poisons," 3rd ed., London: The Pharmaceutical Press, 2004.
Saferstein, Richard, "Forensic Science Handbook," New Jersey: Prentice Hall, 1988.
Scientific Working Group for the Analysis of Seized Drug Recommendation, 6th ed., "Part III A & B, Methods of Analysis/Sampling of Seized Drug for Qualitative Analysis," July 2011

6. Definitions

GC: Gas Chromatography
GC/MS: Gas Chromatography/Mass Spectrometry
Gross Weight:
Net Weight:

7. Supplies, Equipment & Reagents

Supplies

Culture tubes
Syringes with needle
Scissors
Spatula
Pasteur pipette
Tweezers
Weighing dish
Weighing paper
GC vials with Teflon caps

Equipment
Analytical Balance
GC
GC/MS

Reagents
p-dimethylaminobenzaldehyde (p-DMAB)
Lysergic acid diethylamide (LSD)
Lysergic acid methylpropylamide (LAMPA)
Methanol
Chloroform

8. Safety

Due to the potential hazards, appropriate precautions should be taken as necessary. This includes, but is not limited to, the use of fume hoods, gloves, masks and safety glasses. Lab coats are to be worn at all times in the unit, unless performing administrative duties.

9. Reagent Preparation

Anastrozole Standard
Dissolve 2.0mg of anastrozole and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Androstenediol Standard
Dissolve 2.0mg of androstenediol and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Boldenone Undecylenate Standard
Dissolve 4.0mg of boldenone undecylenate and bring to volume with 1.8mL of chloroform. Mix the solution until completely dissolved.

Bolandiol Standard
Dissolve 0.2mg of bolandiol and bring to volume with 0.2mL of methanol. Mix the solution until completely dissolved.

1-Dehydrotestosterone Standard
Dissolve 2.0mg of 1-dehydrotestosterone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Drostanolone Standard
Dissolve 2.0mg of drostanolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Drostanolone Propionate Standard
Dissolve 2.0mg of drostanolone propionate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Estrone Standard

Dissolve 2.0mg of estrone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

17α -Estradiol Standard
Dissolve 2.0mg of 17α–estradiol and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Mesterolone Standard
Dissolve 2.0mg of mesterolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Methandrostenolone Standard
Dissolve 2.0mg of methandrostenolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Methenolone Standard
Dissolve 0.2mg of methenolone and bring to volume with 0.2mL of methanol. Mix the solution until completely dissolved.

Methyldihydrotestosterone Standard
Dissolve 1.0mg of methyldihydrotestosterone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

17α-Methyltestosterone Standard
Dissolve 2.0mg of 17α-methyltestosterone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Nandrolone Standard
Dissolve 2.0mg of nandrolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Namdrolone Decanoate Standard
Dissolve 2.0mg of nandrolone decanoate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Nandrolone Phenpropionate Standard
Dissolve 2.0mg of nandrolone phenpropionate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Norgestrel Standard
Dissolve 2.0mg of norgestrel and bring to volume with 1.8mL of acetone. Mix the solution until completely dissolved.

Ondansetron Hydrochloride Dihydrate Standard
Dissolve 2.0mg of ondansetron hydrochloride dihydrate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Oxandrolone Standard
Dissolve 2.0mg of oxandrolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Oxymethalone Standard
Dissolve 1.0mg of oxymetholone and bring to volume with 1.8mL of chloroform. Mix the solution until completely dissolved.

Stanozolol Standard
Dissolve 4.0mg of stanozolol and bring to volume with 1.8mL of chloroform. Mix the solution until completely dissolved.

Testosterone Standard
Dissolve 2.0mg of testosterone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Testosterone Decanoate Standard
Dissolve 2.0mg of testosterone decanoate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Testosterone Enanthate Standard
Dissolved 2.0mg of testosterone enanthate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Testosterone Phenylpropionate Standard
Dissolve 2.0mg of testosterone phenylpropionate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Testosterone Propionate Standard
Dissolve 2.0mg of testosterone propionate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Trenbolone Acetate Standard
Dissolve 2.0mg of trenbolone acetate and bring to volume with 1.8mL of chloroform. Mix the solution until completely dissolved.

Trenbolone Enanthate Standard
Dissolve 2.0mg of trenbolone enanthate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Cobalt Thiocyanate Reagent
Dissolve 2.0g of cobalt thiocyanate in 100mL of deionized water. Mix the solution until completely dissolved.

Marquis Reagent
Dilute 10mL of 37% formaldehyde solution in 90mL of concentrated sulfuric acid. While stirring, slowly add the concentrated sulfuric acid to the formaldehyde solution. Allow the solution to cool completely.

Froedhde's Reagent
Dissolve 0.5g of sodium molybdate in 100mL of concentrated sulfuric acid Mix the solution until completely dissolved.

Mecke's Reagent
Dissolve 1.0g of selenous acid in 100mL of concentrated sulfuric acid. Mix the solution until completely dissolved.

Dilly Koppanyi Reagent

Dilly
Dissolve 0.1g of cobaltous acetate tetrahydrate in 100mL of methanol. Then add 0.2mL of glacial acetic acid to the solution. Mix the solution until completely dissolved.

Isopropylamine
Dilute 5mL of isoproplyamine in 100mL of methanol. Mix the solution completely.

2.8N Hydrochloric Acid Reagent
Dilute 92.6mL of 12.1N hydrochloric acid in 400mL of deionized water. Mix the solution completely.

Cocaine/Codeine Standard (QC Mix)
Dissolve 10.0 mg of cocaine hydrochloride and 10.0mg of codeine phosphate and bring to volume with 10mL of methanol. Mix the solution until completely dissolved.

10. Procedure

A. Document observations on the Drug Analysis Form noting the number, type (e.g. liquid, powder or tablets), color, viscosity and marking of all items.
B. Document if the sample has been tampered or intact.
C. Obtain the weight of the sample.
    i. For liquid or cream solutions and residual patches, only a gross weight should be documented
    ii. For powders and tablets, both a gross weight and net weight should be documented.
D. Color Tests
    i. The color test consists of five reagents, which are and dilly koppanyi.
    ii. Cobalt thiocyanate, marquis, froehde's, mecke's reagents will be placed in individual wells on a porcelain spot plate.
    iii. Dilly Koppanyi reagent will be placed into a culture tube.
    iv. For tablets or capsules, cut a ¼ of the tablet and crush into a powder.
    v. For liquids, remove about ½ mL of solution using a syringe and place into a culture tube.
    vi. Add a small amount of sample to each well or culture tube and observe if there are any reactions.
    vii. The results will be recorded on the Drug Analysis Form by documenting the actual color/s observed. Negative observations will be recorded by stating no reaction present or no color change.

E. Gas Chromatography
    i. The methanolic extract from section (C) can be used for the GC analysis.

ii. Initiate auto sampler sequence using the Genscan method running a blank solvent between each unknown sample and reference standard/s .
iii. Compare retention time of the each sample with the reference standard/s. Also check the chromatograph to determine if the sample needs to be diluted or concentrated.
iv. Positive GC analysis will be recorded on the Drug Analysis Form by the use of a plus (+). The result is considered positive when the retention time of the sample and the reference standard meet the laboratory criteria and are specified in the notes. Negative observations will be recorded by the use of a negative (-).

F. Gas Chromatography/Mass Spectrometry

i. Perform a preventative maintenance on the instrument by cleaning the injection port and changing the liner.
ii. Confirmatory analysis can be performed using the GC vial from the previous section (D).
iii. Initiate auto sampler sequence using the LSD method running a blank solvent between each unknown sample and reference standard/s.
iv. Compare retention time and ion spectra of the each sample with the reference standard/s.
v. Document the date analyzed and results of the GC/MS onto the MS Tracking Sheet, Drug Analysis Form and Control Card.

11. Documentation

A. All results will be documented on the Drug Analysis Form.
B. All raw data will be generated and filed according to the laboratory policy.
C. A certificate of analysis will be generated for each lab number which will document the results.

12. Attachments